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PRINCIPAL INVESTIGATOR: Ercole L. Cavalieri, D.S.C.

CONTRACTING ORGANIZATION: University of Nebraska Medical Center
Omaha, Nebraska 68198-7835

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6. AUTHOR(S)
Ercole L. Cavalieri, D.S.C.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Nebraska Medical Center
Omaha, Nebraska 68198-7835

E-Mail: ecavalie@unmc.edu

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13. ABSTRACT (Maximum 200 Words)

Treatment of Noble rats with testosterone plus estradiol (E_2) induces prostate carcinomas. We think that estrogens initiate prostate cancer by reaction of catechol estrogen-3,4-quinone (CE-3,4-Q) metabolites with DNA. Formation of depurinating adducts by CE-3,4-Q, which generate apurinic sites in DNA, would be the critical event leading to mutations that initiate prostate cancer. After treatment of rats with CE or CE-3,4-Q, CE metabolites and CE-glutathione (GSH) conjugates were lower in regions where tumors develop and methoxyCE were higher in regions where tumors do not develop. To study the role of CE-Q in initiation of prostate cancer, we are (1) treating rats with E_2 and/or testosterone and analyzing the CE metabolites, CE-GSH conjugates and depurinating CE-DNA adducts in the regions of the prostate by HPLC with electrochemical and mass spectrometric detection; (2) studying in the prostate conversion of testosterone into E_2 and its metabolism; and (3) determining the expression at the mRNA level of four selected enzymes involved in estrogen activation and deactivation in the prostate of rats treated with E_2 and/or testosterone. These studies will provide information critical to understanding the molecular etiology of prostate cancer, identify biomarkers for early detection of susceptibility and lead to development of strategies for prostate cancer prevention.

14. SUBJECT TERMS
estrogens as endogenous tumor initiators; initiation of prostate cancer in Noble rats by estrogens; analysis of estrogen metabolites, conjugates and DNA adducts; expression of estrogen activating and deactivating enzymes

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Introduction

The purpose of this research is to investigate the hypothesis that estradiol (E_2) initiates prostate carcinogenesis and testosterone promotes the process. This is being explored in male Noble rats, which develop prostate tumors when treated with E_2 and testosterone [1]. We think that estrogens are involved in the initiation of prostate cancer by a mechanism that involves oxidation of endogenous 4-catechol estrogen (CE) metabolites to CE-3,4-quinones (CE-3,4-Q). Reaction of CE-3,4-Q with DNA results in tumor initiation as the first step in the events leading to prostate cancer. Formation of depurinating DNA adducts by CE-3,4-Q, which generate apurinic sites in DNA, would be the critical event leading to mutations that initiate the cancer [2]. To study the role of CE-Q in the initiation of prostate cancer, we are (1) treating male Noble rats with E_2 by i.p. injection at various doses and for various times, analyzing the estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts and comparing their levels in the various regions of the prostate [3]; (2) investigating the conversion of testosterone into E_2 in the prostate by analyzing the same compounds in prostate tissues from rats treated with testosterone or testosterone plus the aromatase inhibitor letrozole; and (3) determining the expression of four enzymes involved in the activation and deactivation of estrogens, cytochrome P450 (CYP) 19 (aromatase), CYP1B1, catechol-*O*-methyltransferase (COMT) and quinone oxidoreductase (QOR). The results of these studies will provide information on the relationship between estrogen activation and deactivation in relation to tumor initiation in the prostate.

Body

In the second year of this research project, significant progress has been made on the projected tasks, as detailed in the Statement of Work. The results of the initial studies are reported below. Based on these results, we have modified our proposed studies.

Task 1: Conduct the E_2 dose-response study of CE metabolites, GSH conjugates and DNA adducts.

Noble rats were treated with 0, 16, 32 or 48 mg/kg of E_2 by i.p. injection, and after 3 h the prostate tissues were collected and sent to UNMC for analysis. The HPLC analyses with electrochemical and mass spectrometric detection were conducted. Very few metabolites or conjugates were detected at very low levels. One possible explanation of these results is that the treatment with E_2 severely damaged the prostate, greatly inhibiting E_2 metabolism. In fact, implantation of Noble rats with E_2 produces prostate atrophy [1].

In addition, animals were treated with testosterone by implantation for 2 wk or by i.p. injection of 0 or 52 mg/kg for 6 h (in preparation for Tasks 6 and 7). The prostate tissues were collected and sent to UNMC for analysis. The HPLC analyses with electrochemical and mass spectrometric detection were conducted. The key result is that E_2 was detected in the prostate of rats injected with testosterone, but not in the untreated rats. Additional experiments with testosterone will be conducted based on the results of this study.

Task 4: Analyze the expression of estrogen-metabolizing enzymes in control animals.

Analysis of the four enzymes, CYP19, CYP1B1, COMT and QOR, in control rats was conducted by using prostate tissues from the animals in the experiments described above, control rats injected with solvent or implanted with an empty implant. The normal levels of expression in the prostate of the four enzymes at the mRNA level are shown in Table 1.

Table 1. Expression of CYP19, CYP1B1, COMT and QOR at the mRNA level in regions of the prostat of control rats

	Enzyme, copies of mRNA/μg of total RNA			
Enzyme	CYP19	CYP1B1	COMT	QOR
Dorsolateral prostate	1.60×10^{6a}	0.71×10^{7a}	4.42×10^{11a}	$1.36\ 10^{7} \pm 0.85 \times 10^{7}$
Urethra	$4.31 \times 10^{6} \pm 2.28 \times 10^{6}$	2.00×10^{7a}	$1.74 \times 10^{11} \pm 1.24 \times 10^{11}$	$1.93 \times 10^{7} \pm 1.41 \times 10^{7}$
Ventral prostate	0.98×10^{6a}	2.50×10^{7a}	$0.54 \times 10^{11} \pm 0.46 \times 10^{11}$	$0.23 \times 10^{7} \pm 0.14 \times 10^{7}$

[a]Multiple determinations were made on only two different samples.

Each of the enzymes was expressed at the mRNA level at similar levels in the three areas of the prostate. It is noteworthy that COMT is expressed at much higher levels than the other three enzymes. Expression of the CYP19 and CYP1B1 proteins was determined by the western blot method. Both proteins were detected in the ventral prostate at about twice the levels found in the dorsolateral prostate and urethra.

Task 5: Begin analysis of the expression of estrogen-metabolizing enzymes in E_2-treated animals.

Analysis of the four enzymes, CYP19, CYP1B1, COMT and QOR, in rats treated with E_2 or testosterone (as described in Task 1) was conducted. The levels of expression of the four enzymes at the mRNA level are shown in Tables 2 and 3.

Table 2. Expression of CYP19, CYP1B1, COMT and QOR at the nRNA level in regions of the prostate of rats treated with testosterone

	Enzyme, copies of mRNA/μg of total RNA[a]			
Enzyme	CYP19	CYP1B1	COMT	QOR
Implantation (2 wk)				
Dorsolateral prostate	2.66×10^{6}	4.22×10^{7}	2.64×10^{10}	6.57×10^{6}
Urethra	2.11×10^{6}	2.26×10^{7}	2.93×10^{10}	5.44×10^{6}
Ventral prostate	0.64×10^{6}	0.78×10^{7}	1.72×10^{10}	1.68×10^{6}
Injection (6 h)				
Dorsolateral prostate	1.14×10^{6}	1.37×10^{7}	2.40×10^{10}	5.66×10^{6}
Urethra	0.40×10^{6}	0.83×10^{7}	0.25×10^{10}	0.73×10^{6}
Ventral prostate	1.54×10^{6}	0.78×10^{7}	1.03×10^{10}	0.96×10^{6}

[a]Multiple determinations were made on two different samples.

Table 3. Expression of CYP19, CYP1B1, COMT and QOR at the nRNA level in regions of the prostate of rats injected with E_2

	Enzyme, copies of mRNA/μg total RNA[a]			
Enzyme	CYP19	CYP1B1	COMT	QOR
16 mg E_2/kg				
Dorsolateral prostate	0.80×10^8	4.12×10^8	4.96×10^{10}	6.46×10^7
Urethra	4.63×10^8	-[b]	10.6×10^{10}	12.9×10^7
Ventral prostate	1.96×10^8	2.39×10^8	2.36×10^{10}	2.46×10^7
32 mg E_2/kg				
Dorsolateral prostate	1.48×10^8	7.96×10^8	6.28×10^{10}	4.99×10^7
Urethra	1.55×10^8	6.22×10^8	1.33×10^{10}	1.67×10^7
Ventral prostate	1.11×10^8	2.28×10^8	2.63×10^{10}	1.76×10^7
48 mg E_2/kg				
Dorsolateral prostate	0.36×10^8	1.02×10^8	2.12×10^{10}	2.62×10^7
Urethra	5.79×10^8	3.66×10^8	11.6×10^{10}	20.8×10^7
Ventral prostate	2.69×10^8	6.04×10^8	1.16×10^{10}	1.40×10^7

[a]Multiple determinations from two different samples.
[b]Data were not obtained from this sample.

Implantation of testosterone for two weeks had minimal effects on the expression of the four enzymes at the mRNA level (Table 2), except that CYP19 and CYP1B1 appeared to be induced in the dorsolateral prostate and the level of COMT was consistently reduced. As could be anticipated, expression of the enzymes had changed little 6 h after injection of testosterone, except that the level of COMT was greatly reduced. Treatment with testosterone had no effect on the levels of the CYP19 and CYP1B1 proteins, except that both enzymes seemed to be increased in the ventral prostate two weeks after implantation. The short-term (3 h) effects of injection with E_2 on the expression of the enzymes (Table 3) were questionable. Once again, the levels of COMT were several orders of magnitude greater than that of the other three enzymes, but lower than in the control tissues (Table 1). The other three enzymes appeared to be increased, but this result would have to be repeated for validation.

Based on these results, we have begun a follow-up study to discover whether (1) the treatment with E_2 is destroying the prostate and (2) simultaneous treatment with testosterone can reverse this effect. In this study rats are being treated with E_2 alone for 3 or 6 h, implanted testosterone plus E_2 for 3 or 6 h, implanted testosterone or vehicle alone. The estrogen metabolites, estrogen conjugates or estrogen-DNA adducts will be analyzed. In addition, the expression of CYP19, CYP1B1, COMT and QOR will be determined in the vehicle and implanted testosterone groups. Following this experiment, the effects of testosterone plus the aromatase inhibitor letrozole will be compared to treatment with testosterone alone.

Key Research Accomplishments

1. Groups of rats were treated with E_2 (3 different doses injected for 3 h) or testosterone (implanted for 2 wk or injected for 6 h), the prostates were excised and dissected into the dorsolateral prostate, ventral prostate, and urethra, and the tissues were analyzed for estrogen metabolites, estrogen conjugates and estrogen-DNA adducts by HPLC with electrochemical and mass spectrometric detection.

2. Tissues from the E_2 and testosterone experiments were analyzed for expression of the estrogen-metabolizing enzymes CYP19 (aromatase), CYP1B1, COMT and QOR at the mRNA level and for CYP19 and CYP1B1 at the protein level (testosterone experiment only).

Reportable Research Accomplishments

Singh, S., Bosland, M.C., Cavalieri, E.L. and Rogan, E.L. Effect of treatment with estradiol or testosterone on the expression of CYP19, CYP1B1, COMT and NQO1 in the prostate of male Noble rats. *Proc. Amer. Assoc. Cancer Res.*, #14, 2004.

Conclusions

In this second year, we have analyzed estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts in the regions of rat prostate after treatment with E_2 or testosterone. We have shown that following treatment with testosterone, the prostate contains significant amounts of E_2, which is not present in the prostates of untreated rats. We have determined the expression of four selected estrogen-metabolizing enzymes, CYP19, CYP1B1, COMT and QOR, in the regions of the prostate from control rats and rats treated with E_2 or testosterone. We have shown that all of these enzymes are, indeed, present in the rat prostate.

References

1. Bosland, M.C., Ford, H., and Horton, L. Induction at high incidence of ductal prostate adenocarcinomas in NBL/Cr and Sprague-Dawley Hsd:SD rats treated with a combination of testosterone and estradiol-17β or diethylstilbestrol. Carcinogenesis, 16: 1311-1317, 1995.

2. Cavalieri, E.L., Rogan, E.G. and Chakravarti, D. Initiation of cancer and other diseases by catechol ortho-quinones: A unifying mechanism. Cell & Mol. Life Sci., 59: 665-681 2002.

3. Cavalieri, E.L., Devanesan, P., Bosland, M.C., Badawi, A.F. and Rogan, E.G. Catechol estrogen metabolites and conjugates in different regions of the prostate of Noble rats treated with 4-hydroxyestradiol: Implications for estrogen-induced initiation of prostate cancer. Carcinogenesis, 23: 329-333, 2002.